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Optical detection of choline and acetylcholine based on H_2O_2-sensitive quantum dots

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ABSTRACT

In this paper, we have constructed a simple, rapid and sensitive biosensor for detection of choline and acetylcholine (ACh) based on the hydrogen peroxide (H_2O_2)-sensitive quantum dots (QDs). The detection limit for choline was 0.1 μM and the linear range was 0.1–0.9 μM and 5–150 μM, respectively. The detection limit for ACh was found to be 10 μM and the linear range was 10–5000 μM. The wide linear ranges were shown to be suitable for routine analyses of choline and ACh. Possible mechanism of the fluorescence of QDs quenched by H_2O_2 was an electron transfer (ET) process. The experimental conditions of biosensors were optimized, and anti-interference ability was also presented. We also detected the choline in milk samples and the linear range was 5–150 μM. The detection linear range of ACh in serum was 10–140 μM. Most importantly, the recovery of choline in milk and ACh in serum samples were both close to 99%. The excellent performance of this biosensor showed that the method can be used in practice detection of choline and ACh.

1. Introduction

CdTe quantum dots (QDs) are a new kind of fluorescent nanometer-sized particles of intense research and broad applications (Alivisatos, 2004; Chan and Nie, 1998; Gao et al., 2005; Michalet et al., 2005). Comparing to organic dyes and fluorescent proteins, QDs have unique functional and structural properties, such as size and composition tunable fluorescence emission, large absorption cross-sections, and exceptional brightness and photostability. A lot of researches focus on QDs-enzyme biosensors (Borisov and Wolfbeis, 2008; Ren et al., 2010; Yang et al., 2011) in recent years due to their unique, novel properties and biomedical applications. However, these studies mainly focus on some routine substance such as glucose (Bahshi et al., 2009; Hu et al., 2010; Tang et al., 2008; Xiao et al., 2005). There are few such researches about the detection of choline and acetylcholine which also present important roles in human body (Caudill, 2010; Chawla et al., 1989). Choline is a kind of Vitamin B, which could promote the liver and kidney fat metabolism. It also participates in the synthesis of ACh. ACh is an essential messenger, which is involved in neurotransmission in both the peripheral and central nervous systems. The decrease of this compound makes individuals prone to various nerve disorders including Parkinson's disease, Alzheimer's and multiple sclerosis (Schebb et al., 2008). Furthermore, choline and Acetylcholine-rich foods are very good to patients with Parkinson's disease, Alzheimer's and multiple sclerosis (Dani et al., 2004; Fintelmann and Lindner, 1972; Newhouse et al., 2004). As an essential nutrient for humans (Zeisel, 2000), the choline and acetylcholine were widely added in foods such as milk and health protection foods. So the quantitative determination of choline and acetylcholine is very important in clinical analysis (such as human serum, bile, amniotic fluid, brain extracts and pharmaceutical products), food industry, feed additives and many other fields (Campanella et al., 1988, 1989; Casson and Griffin, 1959; Ueland, 2011).

Many different methods have been used in the determination of choline and acetylcholine, such as electrochemical detection (Bai et al., 2007; Burmeister et al., 2008; Ren et al., 2009; Zhu et al., 2009), colorimetric detection (Takayama et al., 1977), high performance liquid chromatography (HPLC) detection (Kuribayashi et al., 2008; Xiao et al., 1999), fluorescence detection (Bai et al., 2008; Qin et al., 2009; Qin et al., 2010) and chemiluminescence detection (Buiculescu et al., 2010; Burmeister et al., 2008; Dai et al., 2010; Wei et al., 2009). However, the detection ways of the above still have some problems. The electrochemical detection systems required

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complicated modifications of the electrodes and the HPLC detection needed long time and complex operation, etc. The fluorescence and chemiluminescence detection systems were still suffering from the unstable optical signal. These also block the practical application of choline and ACh detection. So it urgently needs to develop a sensitive, precise, simple and convenient detection way for choline and ACh.

In this paper, we have constructed a novel, fast and simple choline and ACh measuring method based on the fluorescence of QDs. The biosensor was composed of H_2O_2-sensitive QDs and enzyme, without any complex processes of functionalization or conjugation. So the whole process from assembly to detection could be finished in about 15 min, which was faster than other methods (Schuvailo et al., 2005). The convenient detection of choline and ACh was based on the fluorescence quenching of the QDs by H_2O_2 which was produced from the enzymatic reaction of choline or ACh. The obtained optical biosensor showed low detection limit, wide linear detection range and good selectivity. The practical detection in simulated samples and practical sample also has been studied and the results showed this biosensor can be used for practical application. This optical analysis method could provide a generic way to analyze numerous H_2O_2-dependent enzymes due to many enzymatic reactions can produce hydrogen peroxide.

2. Experimental

2.1. Materials

AChE (type V-S, from *Electrophorus electricus*, 425.94 U/mg), ChOx (EC 1.1.3.17, from Alcaligenes sp. Lyophilized powder, ~10 U/mg). Choline were obtained from Beijing chemical reagents company. Thioglycolic acid (TGA) was purchased from Alfa Aesar, a Johnson Matthey company. Tellurium powder and cadmium nitrate were purchased from Institute of Tianjin Jinke fine chemicals. The reagents such as choline and cadmium nitrate were of analytical reagent grade. Phosphate buffered saline (PBS) was prepared: 1.02 mM Na_2HPO_4, 6.45 mM KH_2PO_4, pH 7.01. The ultrapure water (0.22 μm) was produced using a Millipore-Q water system.

2.2. Synthesis of CdTe QDs

CdTe QDs were obtained via a method described in the literature with minor modification (Gaponik et al., 2002). Briefly, the CdTe precursor solution was prepared by adding freshly prepared NaHTe solution to a nitrogen-saturated $CdCl_2$ solution at pH 11.5 (adjusted by dropwise addition of 1 M solution of NaOH) in the presence of thioglycolic acid (TGA) as stabilizer. The precursor concentrations were [Cd] = 10 mM, [TGA] = 14 mM, [Te] = 5 mM, respectively. The CdTe precursor solution was heated at 90 °C for 6 h. With the reacting time prolonged, the colour of CdTe precursor solution gradually changed from wine red to orange and then bright red. Then the QDs used in this paper were obtained.

As shown in Fig. S1(A) (see the Supporting Information Fig. S1(A)), the obvious UV–vis absorption peak indicated that the CdTe QDs were closed to monodispersed. The fluorescence emission maximum appeared at 575 nm. The photoluminescence full width at half-maximum was about 47 nm for QDs. The concentration of the QDs was 0.94×10^{-6} M (Yu et al., 2003). The X-ray Diffraction (XRD) patterns of the CdTe QDs are shown in Fig. S1(B) (see the Supporting Information Fig. S1(B)). The peak in the (1 1 1) direction is at 24.4°. The XRD results indicate that QDs have fine nanocrystal structure which is consistent with the reported results (Lee et al., 2008). As shown in Fig. S1(C) (see the Supporting Information Fig. S1(C)), transmission electron microscope (TEM) images also suggest that the CdTe QDs have a narrow size distribution. The average size of the CdTe QDs was 3.0 nm (Yu et al., 2003).

2.3. Instrumentation

Fluorescence measurements were carried out on a Cary Eclipse fluorescence spectrophotometer (Varian, Inc.). The emission spectra were recorded in the wavelength of 500–650 nm upon excitation at 480 nm. The exciting slit and the emission slit were 5 and 5 nm, respectively. The samples for the fluorescence measurements were placed in a 10 mm optical path length quartz fluorescence cuvette.

2.4. Fluorescence experiments

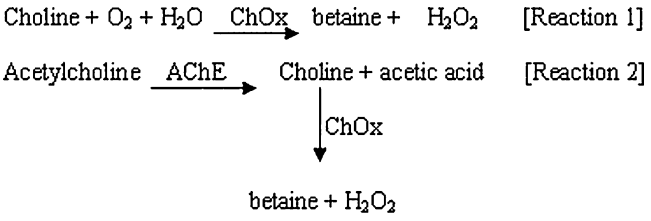
Choline detection procedure was described as follows: 20 μL of QDs was diluted into 400 μL (0.47×10^{-7} M), and then ChOx and different concentrations of choline solution (concentration from 0.1 to 200 μM) were stepwise added in QDs solution within 1 min. The mixture obtained was kept for 10 min before detection.

ACh detection procedure was described as follows: 20 μL of QDs was diluted into 400 μL (0.47×10^{-7} M), and then AChE, ChOx and different concentrations of ACh solution (concentration from 5 to 5000 μM) were stepwise added in QDs solution within 1 min. The mixture obtained was kept for 10 min before detection.

3. Results and discussion

3.1. The quenching effect of the H_2O_2 on CdTe QDs

Recently, some researchers have found that H_2O_2 can efficiently quench the fluorescence of QDs (Mancini et al., 2008). Based on this principle, the biocatalyzed generation of H_2O_2 by oxidases can be used for the development of photochemical biosensors. Furthermore, both ACh and choline can be catalytic oxidation to H_2O_2. The reaction of ChOx generates H_2O_2 as a product was shown in Reaction 1. In addition, the ACh can be catalyzed by AChE to choline and then choline was catalyzed by ChOx to H_2O_2 (shown in Reaction 2). Thus, controlling the properties of QDs quenched by H_2O_2 can realize the detection of ACh and choline.



Firstly, we have experimented for confirming the quenching effect of H_2O_2 on QDs. The fluorescence changes in the presence of variable concentrations of H_2O_2 upon interaction with the QDs for a fixed time interval of 10 min were shown in Fig. S2 (see the Supporting Information Fig. S2). As shown in Fig. S2, the emission maximum of QDs was observed to be quenched by H_2O_2. With the increasing concentrations of H_2O_2, the emission of QDs decreased gradually. The obvious fluorescence influence of QDs indicated a strong interaction between QDs and H_2O_2.

3.2. Fluorescence detection of choline based on H_2O_2-sensitive QDs

Fig. 1A shows the time-dependent fluorescence quenching of the QDs upon treatment of the QDs with 500 U/L ChOx and 150 μM choline. As the time was prolonged, the quenching effect of the QDs intensified. The result indicated that the QDs had been quenched by

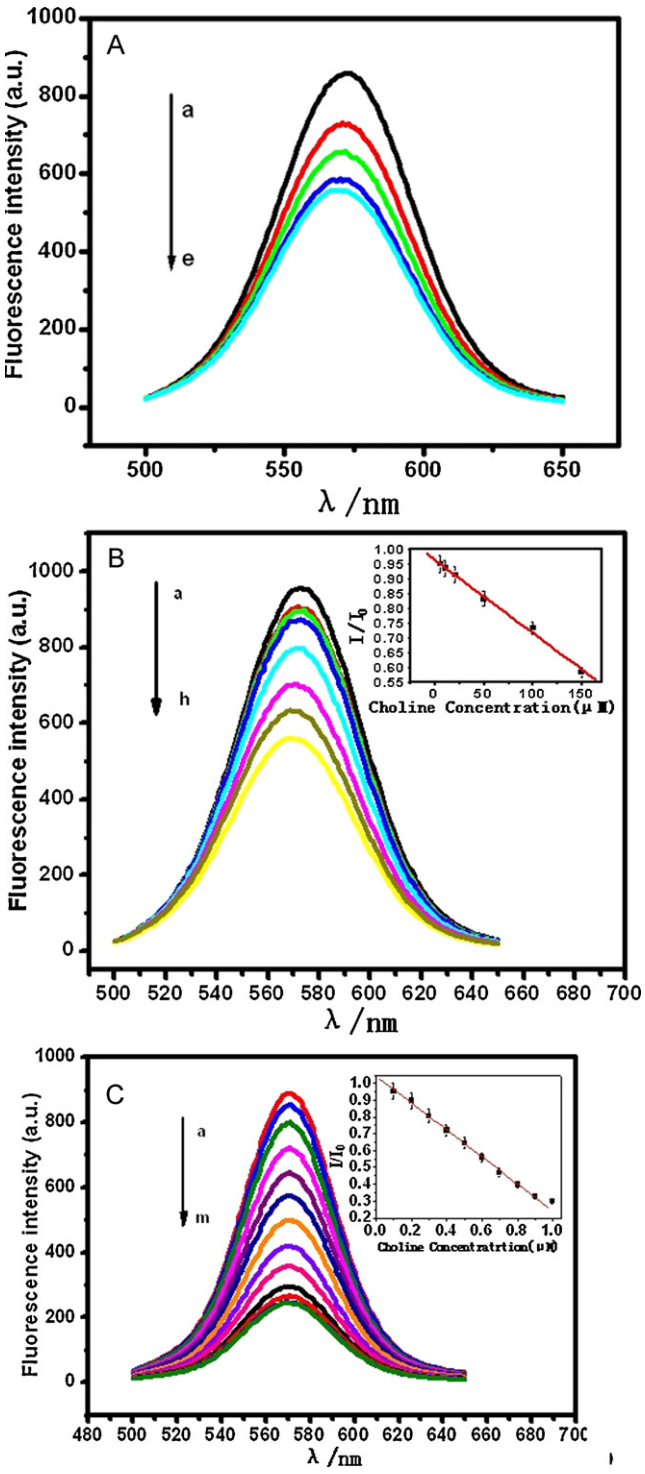


Fig. 1. (A) Time-dependent fluorescence changes of QDs in the presence of 500 U/L ChOx and 150 μM choline. a–e: 1 min, 3 min, 5 min, 7 min, 10 min; (B) Emission spectra of QDs in the presence of 500 U/L ChOx and varying concentrations of choline. a–h: 0, 5 μM, 10 μM, 20 μM, 50 μM, 100 μM, 150 μM, 200 μM. The inset displays plots of the relative fluorescence intensity of QDs versus the concentration of choline. (C) Emission spectra of QDs in the presence of 1250 U/L ChOx and varying concentrations of choline. a–m: 0, 0.1 μM, 0.2 μM, 0.3 μM, 0.4 μM, 0.5 μM, 0.6 μM, 0.7 μM, 0.8 μM, 0.9 μM, 1.0 μM, 1.1 μM, 1.2 μM. The inset displays plots of the relative fluorescence intensity of QDs versus the concentration of choline.

H_2O_2 which generated during the ChOx catalytic reaction. Fig. 1B and C shows the degree of quenching effect of the fluorescence of QDs upon interaction with different concentrations of choline and ChOx (500 U/L in Fig. 1B and 1250 U/L in Fig. 1C) for a fixed time interval of 10 min. As the concentration of choline increased, the quenching effect of the QDs was enhanced. Calibration curve in the inset showed the optical analysis of different concentrations of choline for a fixed interval of 10 min. The plots exhibited a good linear relationship in high concentration (5–150 μM, shown in Fig. 1B) with 500 U/L ChOx. When we added 1250 U/L ChOx in the detection system, the linear relationship was in low concentration (0.1 μM–0.9 μM, shown in Fig. 1C). The detection limit was 0.1 μM which was lower than some other detection methods (Kudo et al., 2007; Shimomura et al., 2009). The results showed above proved that the developed choline biosensor exhibited a rather higher sensitivity and proper detection range which can realize the detection of choline both in low and high concentration.

3.3. Fluorescence detection of ACh based on H_2O_2-sensitive QDs

Fig. 2A shows the time-dependent fluorescence quenching of the QDs upon treatment of the QDs with 2500 U/L ChOx, 5000 U/L AChE and 80 μM ACh. As the time was prolonged, the quenching

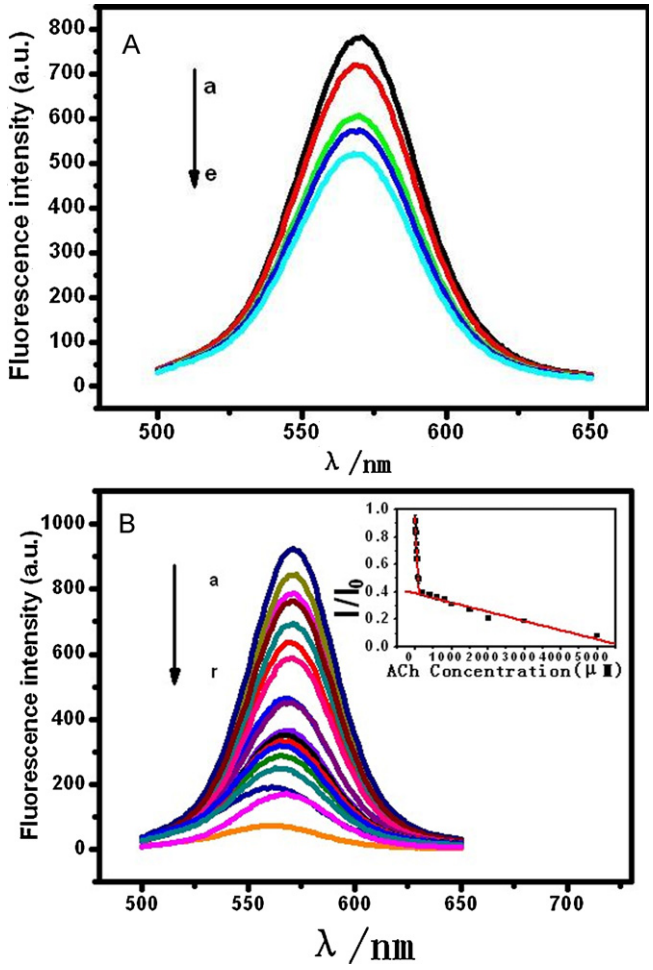
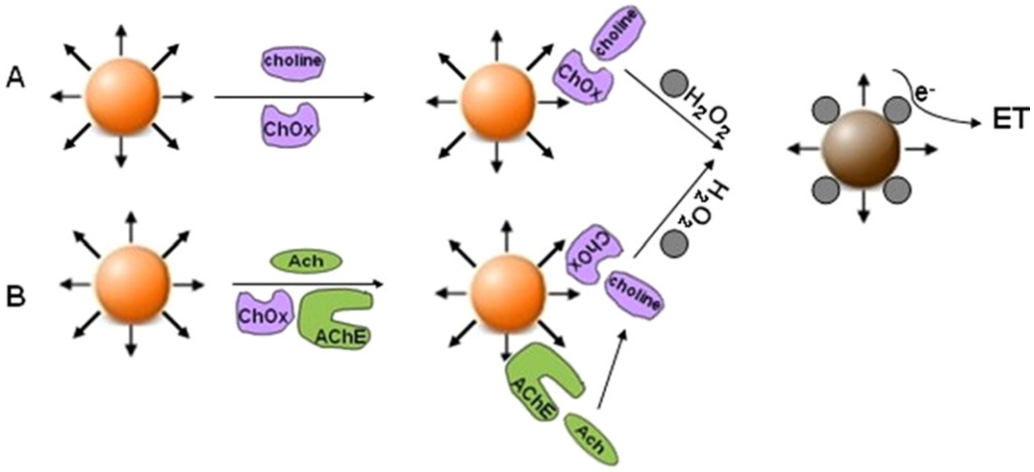


Fig. 2. (A) Time-dependent fluorescence changes of QDs in the presence of 2500 U/L ChOx, 5000 U/L AChE and 80 μM ACh. a–e: 1 min, 3 min, 5 min, 7 min, 10 min; (B) Emission spectra of QDs in the presence of 2500 U/L ChOx, 5000 U/L AChE and varying concentrations of ACh. a–r: 0; 10 μM; 20 μM; 30 μM; 40 μM; 50 μM; 60 μM; 80 μM; 100 μM; 200 μM; 400 μM; 600 μM; 800 μM; 1000 μM; 1500 μM; 2000 μM; 3000 μM; 5000 μM. The inset displays plots of the relative fluorescence intensity of QDs versus the concentration of ACh.



Scheme 1. Schematic principle for assay of (A) choline and (B) ACh biosensor.

effect of the QDs intensified. Fig. 2B shows the degree of quenching of the fluorescence of QDs upon interaction with 2500 U/L ChOx, 5000 U/L AChE and different concentrations of ACh for a fixed time-interval of 10 min. As the concentration of ACh increased, the quenching of the QDs was enhanced. Calibration curve in the inset were the plots of the optical analysis of different concentrations of ACh by the quenching of the emission maximum of the H_2O_2-dependent QDs. The results exhibited a good linear relationship in the range of 10–200 μM and 200–5000 μM with different slope, and the slope of low concentration (10–200 μM) was bigger than the high concentration (200–5000 μM). This was much wider than what observed in electrochemical detection systems (Emteborg et al., 1997). It was also comparable to the colorimetric method (Barak and Tuma, 1979), HPLC method (Holmes-McNary et al., 1996; Karashi and Maruyama, 1993), and capillary electrophoresis (CE) (Zhang et al., 2001). The limit of ACh detection was 10 μM. The results showed that the ACh biosensor had lower detection limit and proper linear range. During the detection of ACh, the addi-

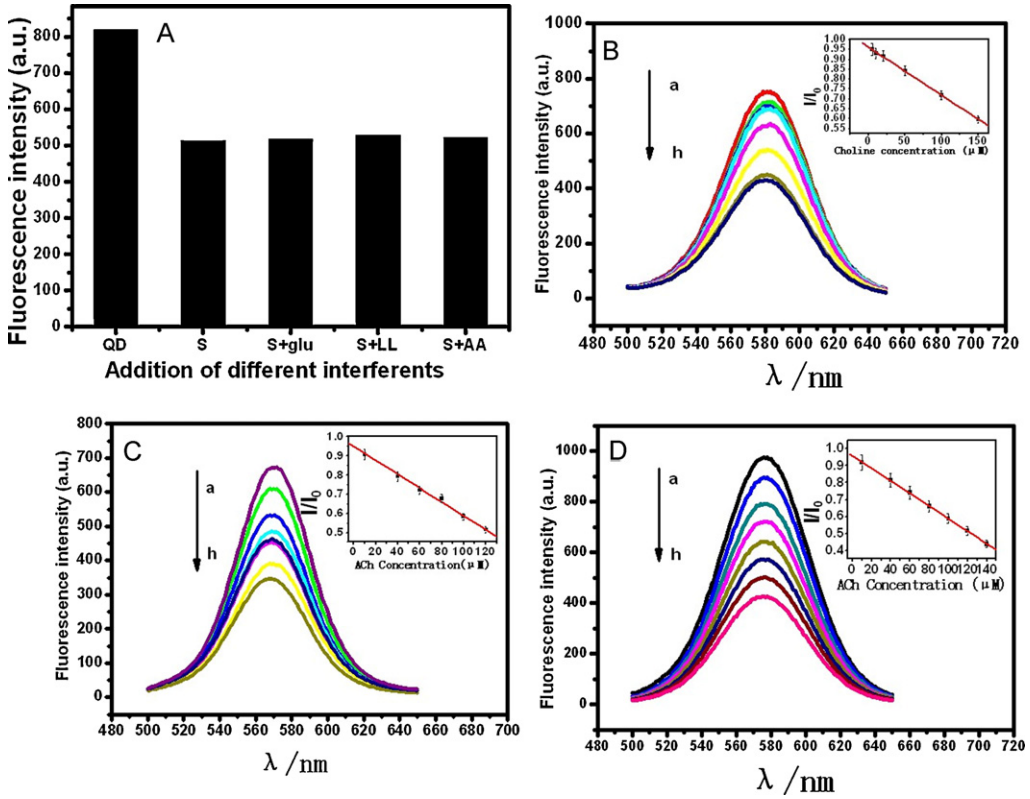


Fig. 3. (A) Fluorescence changes of CdTe QDs in the detection system (S), the coexistence of the detection system with 10 mM glucose (S + glu), 20 mM L-lactic acid (S + LL) or 100 μM ascorbic acid (S + AA), respectively. (B) Emission spectra of QDs in milk with the presence of 1250 U/L ChOx and varying concentrations of choline. a–h: 0; 5 μM; 10 μM; 20 μM; 50 μM; 100 μM; 150 μM; 200 μM. The inner shows the linear plot that reveals a range of 5–150 μM. (C) Emission spectra of QDs in phosphate buffer (pH 7) with the presence of 1250 U/L ChOx, 5000 U/L AChE and varying concentrations of ACh. a–h: 0; 10 μM; 40 μM; 60 μM; 80 μM; 100 μM; 120 μM; 140 μM. The inset displays plots of the relative fluorescence intensity of QDs versus the concentration of ACh. (D) Emission spectra of QDs in the serum with the presence of 1250 U/L ChOx, 5000 U/L AChE, and varying concentrations of ACh. a–h: 0; 10 μM; 40 μM; 60 μM; 80 μM; 100 μM; 120 μM; 140 μM. The inset displays plots of the relative fluorescence intensity of QDs versus the concentration of ACh.

Table 1
Choline detection in milk sample.

Milk sample	Choline added concentration (μM)	Choline found concentration (μM)	RSD (%, $n = 5$)	Recovery (%)
Sample 1	20.00	19.66	1.36	98.3
Sample 2	50.00	49.86	2.54	99.7
Sample 3	100.00	99.37	1.06	99.4
Sample 4	150.00	148.86	0.81	99.2

Table 2
ACh detection in serum sample.

Serum sample	ACh added concentration (μM)	ACh found concentration (μM)	RSD (%, $n = 5$)	Recovery (%)
Sample 1	60.00	58.01	2.52	96.7
Sample 2	80.00	80.02	1.73	100.0
Sample 3	100.00	100.06	1.24	100.0
Sample 4	120.00	120.02	0.52	100.0

tion amount of ChOx was less than AChE, so ChOx kinetics was the limiting factor for the QDs sensor response to ACh (Mitchell, 2004). Therefore we compared K_m' (calculated to be 0.20 mM by Lineweaver-Burke plot) of our ACh sensor with the reported K_m (0.87 mM) (Ohta-Fukuyama et al., 1980). The result indicated that the enzyme remained high activity in the QDs solution.

3.4. Study the quenching mechanism of enzyme reaction on the fluorescence of QDs

The control experiments of ACh and choline detection system (see supporting information Fig. S3) show the fluorescence of CdTe QDs changed slightly in the presence of AChE, ChOx, ACh or choline, respectively. Furthermore, the fluorescence intensity of the QDs showed intensified slightly in the presence of choline. The results proved that the formations of H_2O_2 (shown in Fig. S1) are essential to quench the emission maximum of the QDs. Some papers have reported that the quenching of the fluorescence of the QDs by the H_2O_2 is presumable by an electron transfer (ET) process for modulating the surface chemistry of CdTe QDs (Cao et al., 2008; Gill et al., 2005; Wang et al., 2009). As electron/hole trapped on QDs is a good electron donor/acceptor, H_2O_2 generated in the enzyme reaction may cause the formation of non-fluorescent QDs ion (Bae et al., 2004; Sandros et al., 2005; Wang et al., 2001). In addition, since H_2O_2 is very active, it is prone to react with thiolation. H_2O_2 originally diffused to the surface of QDs by random chemical adsorption or electrostatic force, and caused thiolation fell off from the surface of QDs by affecting the surface chemical bond of QDs (Hu et al., 2010; Shiang et al., 2009). In our detection system, there were complicated factors showed above that lead to the quenching effect of QDs by H_2O_2.

The possible schematic illustration of choline and ACh biosensor are shown in Schematic 1A and B, respectively. During the detection of choline, ChOx diffused to the surface of CdTe QDs and catalyzed choline to produce H_2O_2 (see in Eq. 1), and then the H_2O_2 make electron transfer (ET) process with CdTe QDs. The fluorescence of the QDs was quenched. With the increasing of choline, the quenching effect of the QDs has been enhanced, because the produce H_2O_2 was increased. So choline can be detected based on this principle and the proposed mechanism (shown in Scheme 1A). The process of ACh detection was same as what had been stated above due to ACh could be catalyzed by AChE and generated choline (see in Eq. 2) and the mechanism of ACh detection was showed in Scheme 1B.

3.5. The anti-interference ability

The interference study is one of the important evaluations of the biosensor. Fig. 3A shows that significant fluorescence declined after the introduction of 2500 U/L ChOx, 5000 U/L AChE and 100 μM ACh in the CdTe QDs solution (see "S" in Fig. 3A). Then the additions of 10 mM glucose (see "S + glu" in Fig. 3A), 20 mM L-lactic acid (see "S + LL" in Fig. 3A), and 100 μM ascorbic acid (see "S + AA" in Fig. 3A) did not cause any observable changes of the fluorescence intensities of the QDs in the mixture, respectively. The results proved that the established method can provide selective detection of the activity level of enzyme in the complicated system and can give credible fluorescence signal though the interfering species reach high concentrations.

3.6. The preliminary study on application

Milk is an important part of the diet in people's daily life. As people come to realize the important function of choline in human body, adding choline in the milk become a boom. However, the determination of choline in milk still evoked some problems, such as easily interfered, required complicated manipulation and expensive instrumentation. Hence, there urgently needs a simple and accurate method to detect choline in milk sample. The choline biosensor in this paper had been tested to detect choline in milk and proved to be a feasible method. The solution samples were mixed with QDs, milk (bought in the market), 1250 U/L ChOx and different concentration choline. And, every samples were diluted into 400 μL by milk. The quenching effect of the QDs intensified as the enzymic catalytic reaction time was prolonged (see from the supporting information Fig. S4). As Fig. 3B shown, the fluorescence intensity of the QDs gradually decreased with the increased of choline. The linear calibration range was 5–150 μM.

The recovery of choline in milk was also calculated and shown in Table 1. From Table 1, we can see that the data determined by choline biosensor have a good agreement with the added concentration of choline in the milk sample. It proved that the choline biosensor has great potential in application of choline detection on the food inspection.

The detection of ACh mostly used in clinical analysis of body fluid. So we firstly tested ACh in phosphate buffer (pH 7) (see in Fig. 3C). The solution samples were mixed with QDs, 1250 U/L ChOx, 5000 U/L AChE and different concentration ACh (concentration of 10–140 μM). As reaction time prolonged, the quenching effect of

the QDs intensified (see from the supporting information Fig. S5). The fluorescence changes of CdTe QDs and the liner relationship were shown in Fig. 3C (10–120 μM).

We also tested ACh in serum solution to investigate the possible application of ACh biosensor in clinical analysis. The solution samples were mixed with QDs, serum, 1250 U/L ChOx, 5000 U/L AChE, and different concentration ACh (concentration of 10–140 μM). And, samples were diluted into 400 μL by serum.

The quenching effect of the QDs intensified as the enzymic catalytic reaction time was prolonged (see from the supporting information Fig. S6). With the increased of ACh, the degree of fluorescence quenching of QDs increased gradually. The linear calibration range was 10–140 μM (Fig. 3D). The recovery of the ACh in serum was shown in Table 2. From Table 2, we can see that the data determined by ACh biosensor have a good agreement with the added concentration of ACh in the serum sample. It proved that the ACh biosensor is appropriate for the practical application.

4. Conclusions

In this paper, the H_2O_2-sensitive QDs have been successfully applied to develop new fluorescent biosensors for sensitive and selective detection of choline and ACh, respectively. This method provides a new feasible and simple way to detect choline and ACh combining the advantages of QDs and specificity in enzymic catalytic reaction. The biosensor showed relatively rapid response, high sensitivity, broad linear range, low detection limit and good reproducibility. This method can be widely used in clinical analysis, pharmaceuticals, food industry, and so on. Moreover, this work presents a feasible approach for further research in detecting other kinds of substrate which can be catalyzed to generate H_2O_2, such as, glucose, cholesterol, uric acid, and so on.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.06.041.

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